

What is claimed:

1. A method for purifying a tagged protein from a protein preparation, comprising:
 - (a) concentrating the tagged protein in the protein preparation with a negatively charged capture support, comprising the steps of:
 - (i) contacting the protein preparation with the capture support;
 - (ii) washing the capture support with a capture support washing buffer of low ionic strength to remove interfering molecules but not the tagged protein from the capture support; and
 - (iii) eluting the tagged protein from the capture support with a capture support eluting buffer of high ionic strength;
 - (b) purifying the tagged protein from the eluate of step (a) (iii) with a tag-specific affinity support comprising the steps of:
 - (i) contacting the eluate of step (a) (iii) with the tag-specific affinity support;
 - (ii) washing the affinity support with affinity support washing buffer of low ionic strength to remove some impurities but not the tagged protein from the affinity support; and
 - (iii) eluting the tagged protein from the affinity support with an affinity support eluting buffer.
2. The method of claim 1, wherein the capture support washing buffer and the affinity support washing buffer comprise an ionic strength equivalent to about 50 mM to about 150 mM salt equivalent.
3. The method of claim 2, wherein the capture support eluting buffer comprises an ionic strength equivalent to at least about 500 mM salt equivalent.
4. The method of claim 3, wherein the capture support is applied to a column before or after contacting with the protein preparation.

5. The method of claim 3, wherein the affinity support is applied to a column before or after contacting with the eluate of the capture support.
6. The method of claim 3, wherein the negatively charged capture support comprises a
5 polyanion immobilized on a solid support.
7. The method of claim 6, wherein the polyanion comprises one or more compounds selected from the group consisting of heparin, heparin sulfate, dextran sulfate, chondroitin sulfate, polyuronic acid, hyaluronic acid, Dermatan Sulfate, Alginic acid, and
10 carboxymethylcellulose.
8. The method of claim 7, wherein the polyanion is heparin.
9. The method of claim 3, wherein the tagged protein is a polyhistidine-tagged protein,
15 and wherein the affinity support comprises an immobilized metal affinity chromatography support.
10. The method of claim 9, wherein the immobilized metal affinity chromatography support comprises nickel nitrilotriacetic acid, and wherein the affinity support eluting
20 buffer comprises at least 50mM imidazole.
11. The method of claim 10, wherein the polyhistidine-tagged protein is a 6x histidine tagged cytokine with a four-helix bundle motif.
- 25 12. The method of claim 3, wherein the tagged protein is an Fc-tagged protein, and wherein the affinity support comprises one or more from the group consisting of protein A, protein G, and an antibody-specific affinity matrix.
13. The method of claim 12, wherein the affinity support eluting buffer is a non-
30 denaturing buffer with a pH range between about pH 7.0 and about pH 8.0.

14. The method of claim 13, wherein the Fc-tagged protein is an Fc-tagged cytokine with a four-helix bundle motif.

15. The method of claim 13, wherein the affinity support comprises protein A and/or protein G, and wherein the affinity support eluting buffer comprises at least 4 molar MgCl₂.

16. The method of claim 13, wherein the affinity support comprises an antibody-specific affinity support, and wherein the affinity support eluting buffer comprises 50% ethylene glycol.

17. A method for purifying a polyhistidine-tagged cytokine with a four-helix bundle motif from a protein preparation, comprising:

(a) concentrating the tagged protein in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, comprising the steps of:

(i) contacting the protein preparation with the capture support;

(ii) washing the capture support with a capture support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M to remove interfering molecules but not the tagged protein from the capture support; and

(iii) eluting the tagged protein from the capture support with a capture support eluting buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M;

(b) purifying the tagged protein from the eluate of step (a) (iii) with a tag-specific affinity support, wherein the affinity support comprises nickel nitrilotriacetic acid immobilized on a solid support, comprising the steps of:

(i) contacting the eluate of step (a) (iii) with the affinity support;

(ii) washing the affinity support with affinity support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 1 M

to remove some impurities but not the tagged protein from the affinity support; and

(iii) eluting the tagged protein from the affinity support with an affinity support eluting buffer comprising at least 50 mM imidazole.

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18. A method for purifying an Fc-tagged protein from a protein preparation, comprising:

(a) concentrating the tagged protein in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, comprising the steps of:

10 (i) contacting the protein preparation with the capture support;

(ii) washing the capture support with a capture support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M to remove interfering molecules but not the tagged protein from the capture support; and

15 (iii) eluting the tagged protein from the capture support with a capture support eluting buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M;

(c) purifying the tagged protein from the eluate of step (a) (iii) with a tag-specific affinity support, wherein the affinity support comprises protein A and/or protein G immobilized on a solid support, comprising the steps of:

20 (i) contacting the eluate of step (a) (iii) with the affinity support;

(ii) washing the affinity support with affinity support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M to remove some impurities but not the tagged protein from the affinity support; and

25 (iii) eluting the tagged protein from the affinity support with an affinity support eluting buffer, comprising at least 4 molar $MgCl_2$ at a pH range between about pH 7.0 and about pH 8.0.

30 19. A method for purifying an Fc-tagged protein from a protein preparation, comprising:

(a) concentrating the tagged protein in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, comprising the steps of:

- (i) contacting the protein preparation with the capture support;
- 5 (ii) washing the capture support with a capture support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M to remove interfering molecules but not the tagged protein from the capture support; and
- (iii) eluting the tagged protein from the capture support with a capture support eluting buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M;

10 (b) purifying the tagged protein from the eluate of step (a) (iii) with a tag-specific affinity support, wherein the affinity support comprises an antibody-specific affinity support, comprising the steps of:

- 15 (i) contacting the eluate of step (a) (iii) with the affinity support;
- (ii) washing the affinity support with affinity support washing buffer of an ionic strength equivalent to a concentration of about 50 mM to about 2 M to remove some impurities but not the tagged protein from the affinity support; and
- 20 (iii) eluting the tagged protein from the affinity support with an affinity support eluting buffer with a pH range between about pH 7.0 and about pH 8.0.

20. A kit for the capture and purification of a tagged protein comprising in separate
25 containers a negatively charged capture support, and a tag-specific affinity support.

21. The kit of claim 20, wherein the capture support comprises heparin immobilized on a solid support.

22. The kit of claim 21, wherein the tagged protein is a polyhistidine-tagged protein, and wherein the affinity support comprises nickel nitrilotriacetic acid immobilized on a solid support.

5 23. The kit of claim 21, wherein the tagged protein is an Fc-tagged protein, and wherein the affinity support comprises protein A and/or protein G immobilized on a solid support.

24. The kit of claim 21, wherein the kit further comprises one or more of the following: a capture support washing buffer, a capture support eluting buffer, an affinity support 10 washing buffer, an affinity support eluting buffer, and instructions for using the kit.